Section III (Remarks)

A. Summary of Amendments to the Application

By the present Amendment, claims 1, 4, 7, 9, 13, 15 and 46 have been amended and claims 18-45 and 50-61 have been cancelled without prejudice. Claims 2, 5, 8, 14 and 62 were previously cancelled. No new matter within the meaning of 35 U.S.C. §132(a) has been introduced by the foregoing amendments.

Claim 4 has been amended at the examiner's request, to properly reference SEQ ID NO: 2 of the Sequence Listing. Claims 9 and 15 have been amended at the examiner's request to include sequence identifiers for the tetrapeptides recited therein. Claims 1, 7, 13 and 46 have been amended to clarify structural and functional characteristics of the fusion protein, as discussed with the examiner in the Interview on April 16, 2010. Specifically, the amended claims recite that the protein of interest or target protein is linked to the C-terminus of the subtilisin prodomain protein (Specification, p. 6, lines 19-22; p. 13, lines 1-14; Example 5), the formation of a stable complex between the subtilisin prodomain protein and the subtilisin or variant (Specification, p. 6, lines 1-5; p. 13, lines 10-14; p. 15, line 31 to page 16, line 3), the cleavage of the protein of interest or target protein (Specification, p. 7, lines 20-28; p. 8, lines 1-6; p. 15, lines 15-20; Example 1), and the retention of the bound subtilisin prodomain protein – subtilisin/variant complex following cleavage of the protein of interest or target protein (Specification, p. 15, line 31 to page 16, line 3; page 23, lines 11-16; Example 6). Claim 16 has been amended to properly depend from amended claim 13.

The specification has been amended, at the Examiner's request, to include sequence identifiers for SEQ ID NOs: 9-13, to amend a typographical error referring to SEQ ID NO: 2 and to correct the misspelled word "Streptococcal" at page 25. Additionally, new sequence identifier SEQ ID NO: 14 for amino acid sequence FRAM was added to the Sequence Listing and the paragraph at page 22, lines 2-11 was amended accordingly. No additional changes have been made to the specification by the present Response.

The amendments made herein are fully consistent with and supported by the originally-filed disclosure of this application.

B. Comments Regarding April 16, 2010 Interview

Applicant thanks Examiner Moore for granting the interview conducted on April 16, 2010. Applicant received the Interview Summary mailed April 19, 2010 and acknowledges the accuracy of the Summary provided therein.

The examiner's attention is respectfully drawn to Section II above, where claims 1, 7, 13 and 46 have been amended to "clearly indicat[e]…that a fusion partner could be cleaved from a subtilisin prodomain…" (Interview Summary mailed April 19, 2010.)

C. Revised Sequence Listing

In Response to the Examiner's request, a revised Sequence Listing has been prepared and is submitted concurrently with the present Response via EFS filing. The Revised Sequence Listing includes the five tetrapeptide sequences identified by the examiner. The Revised Sequence Listing further includes a sixth tetrapeptide sequence not identified by the examiner, but present at page 22, line 2 of the specification. The sequences included in the Revised Sequence Listing are: SGIK (identified in the revised Sequence Listing as SEQ ID NO: 9), FKAM (identified in the revised Sequence Listing as SEQ ID NO: 10), FKAY (identified in the revised Sequence Listing as SEQ ID NO: 11), FKAF (identified in the revised Sequence Listing as SEQ ID NO: 12), AHAY (identified in the revised Sequence Listing as SEQ ID NO: 13) and FRAM (identified in the revised Sequence Listing as SEQ ID NO: 14).

The revised Sequence Listing is accompanied by a Statement of Identity, providing that the paper copy and computer readable form are identical. Entry of the Revised Sequence Listing into the record of the application is respectfully requested.

D. Objection to the Specification

In the Final Office Action mailed March 23, 2010, the examiner objected to the disclosure because of various informalities identified by the examiner. The examiner's attention is respectfully drawn to Section I above, where all informalities identified by the examiner have been remedied by amendments to the specification. No new matter has been added. Withdrawal of the objection is respectfully requested.

E. Claim Objection

In the Final Office Action mailed March 23, 2010, the examiner objected to claim 4, as the claim referred to SEQ ID NO: 1 as the 77-amino acid sequence of the subtilisin prodomain. As noted by the examiner, in the Sequence Listing, the subtilisin prodomain is provided with the sequence identifier SEQ ID NO: 2. Claim 4 has been amended to recite SEQ ID NO: 2, corresponding with the sequence identifiers provided in the Sequence Listing. No new matter has been added by this amendment. Withdrawal of the objection is respectfully requested.

F. Discussion regarding 35 U.S.C. §112 – written description

In the April 16, 2010 Interview Examiner Moore indicated that amendment of the claims to include recitation of the cleavage and binding characteristics might raise issues under 35 U.S.C. §112. It is respectfully submitted that the amended claims fully comply with 35 U.S.C. §112, first paragraph written description requirements for the following reasons.

It is well established that "[t]o satisfy the written description requirement, a patent specification must describe the claimed invention in sufficient detail that one skilled in the art can reasonably conclude that the inventor had possession of the claimed invention." (MPEP 2163, citing *Moba, B.V. v. Diamond Automation, Inc.*, 325 F.3d 1306, 1319, 66 USPQ2d 1429, 1438 (Fed. Cir. 2003); *Vas-Cath, Inc. v. Mahurkar*, 935 F.2d at 1563, 19 USPQ2d at 1116.)

While "[t]he claimed invention as a whole may not be adequately described where an invention is described solely in terms of a method of its making coupled with its function and there is no described or art-recognized correlation or relationship between the structure of the invention and its function" (MPEP 2163), that is not the case here.

Applicant's claimed fusion protein is specified in both structural and functional terms. As recited in claim 7, the structural elements of the fusion protein are: 1) a target protein (or protein of interest) and 2) a subtilisin prodomain protein, where the target protein is structurally linked to the C-terminus of the subtilisin prodomain protein and where the subtilisin prodomain protein is structurally modified to exhibit an increased affinity for subtilisin or a variant thereof. Functionally, the fusion protein is subject to cleavage by the subtilisin or variant thereof, in order to structurally release the target protein, with the subtilisin prodomain protein remaining bound to the subtilisin or variant thereof. Claims 1, 13 and 46 contain similar structural and functional limitations.

As set forth in Section IIIA, above, the amendments to the claims are well supported in the specification, which reflects the "...described or art-recognized correlation or relationship between the structure of the invention and its function..."

The functional characteristic that the subtilisin prodomain protein remains bound to the subtilisin or variant thereof following cleavage of the target protein/protein of interest, is directly correlated to the structural characteristic of the modification(s) to the subtilisin prodomain protein. As a direct result of such modification(s), the subtilisin prodomain protein binds to the subtilisin or variant thereof with a Kd of 10nM or less. It has been repeatedly emphasized in the prosecution history of this application that such a Kd is indicative of a complex that will bind and remain bound. Such Kd is structural characteristic of the subtilisin prodomain protein when modified as described in applicant's specification. The application amply and adequately describes modifications that would result in a Kd of 10nM or less. Exemplary modifications to the subtilisin prodomain protein are provided, for example, at page 19, line 34 to page 20, line 19, in Table 1 of Figure 3 and in the Examples.

With regard to the functional characteristic of the subtilisin being effective to cleave the protein of interest from the subtilisin prodomain protein, it is well established that subtilisin protease cuts preferentially and efficiently at the cleavage site between the target protein/protein of interest and the subtilisin prodomain protein. Furthermore, the claims have been amended to recite the structural orientation of the elements of the fusion protein, where the target protein/protein of interest is structurally linked to the C-terminus of the subtilisin prodomain protein. This structural limitation, as graphically illustrated in the specification at page 8, line 10, is a structural limitation that supports selective cleavage of the fusion protein.

Accordingly, though the amended claims contain functional limitations, those functions possess a correlation with specific structural limitations recited in the claims. For the foregoing reasons, the amended claims fully satisfy the 35 U.S.C. §112, first paragraph requirements for adequate written description.

G. Rejection of Claims Under 35 U.S.C. §103

In the Final Office Action mailed March 23, 2010 the examiner raised a new rejection of claims 1, 3, 4, 6, 7, 11-13, 17 and 46-49 under 35 U.S.C. §103(a) as being unpatentable over Ruan et al.

1999 and U.S. Patent No. 7,531,325 (Van Rooijen et al.) in view of Grøn et al. 1996 and Grøn et al. 1992. As noted by the examiner, all of these references are of record, but they are applied in a combination that forms a new basis for rejection.

Ruan et al. 1999 does demonstrate, as asserted by the examiner,

> "...the design and preparation of a nucleic acid construct comprising a coding region for a variant subtilisin prodomain region, termed proR9, that possesses the binding affinity, expressed as a dissociation constant of '10nM or less'...[and a substitution] that contributes to an increased binding affinity for subtilisin as compared to a subtilisin prodomain lacking the substitution..." (Final Office Action mailed March 23, 2010, p. 4-5.)

The examiner also correctly notes that:

> "Ruan et al., 1999, do not, however, teach or suggest the preparation of a nucleic acid construct that will provide a variant subtilisin prodomain proR9 as an amino-proximal, or a carboxyl-proximal, fusion partner of another fusion partner, such as a peptide hormone, in a fusion protein, or the recombinant production and isolation of such a fusion protein." (Final Office Action mailed March 23, 2010, p. 5.)

In the April 16, 2010 Interview with Examiner Moore, it was agreed that "...claims clearly indicating that a fusion partner could be cleaved from a subtilisin prodomain could distinguish over the combination citing teachings of Ruan et al. 1999, since Ruan et al., 1999 did not employ a catalytically active subtilisin." (Interview Summary mailed April 19, 2010.) Claims 1, 7, 13, and 46 have been so amended to distinguish over the cited Ruan et al., 1999 reference.

In the Final Office Action mailed March 23, 2010, Van Rooijen et al. was cited:

> "...for teaching the preparation of polynucleotides encoding fusion polypeptides comprising any of several, diverse, polypeptide fusion partners, including hormones according to claims 11 and 16 herein, fused to modified serine protease prodomains wherein such modified prodomains have improved affinity for the cognate protease as shown by increased efficiency of production of the cleaved fusion partner, relative to the efficiency provided by an unmodified prodomain, and further teach the use of the modified prodomain as a separable component for purification of desired fusion partners, including hormones, by affinity chromatography, as well as the use of various host cells for recombinant production of fusion polypeptides, including several host cells of claim 17 herein." (Final Office Action mailed March 23, 2010, p. 5.)

It is noted that where Van Rooijen et al. describe affinity chromatography (col. 12, ll. 43-55) and purification of the fusion protein, it is the entire fusion protein that is selectively bound to antibodies raised against the pro-peptide sequence, which "results in selective retention of the fusion protein." Van Rooijen et al. fails to provide any showing or evidence that the modified

prodomain is a separable component in such affinity chromatography process.

The examiner has contended that:

> "[i]t would have been obvious to one of ordinary skill in the art at the time the invention was made to substitute a nucleic acid construct of claims 1, 3, 4, 6, 12, and 46-49 comprising a nucleic acid sequence encoding a fusion polypeptide that comprises the stabilized subtilisin BPN' prodomain proR9 of Ruan et al., 1999, which has a lysine at its P3 position, for the serine protease prodomain of Van Rooijen et al. in a fusion to the amino terminus of a commercially or pharmaceutically important fusion partner, such as the hormone of Van Rooijen et al…" (Final Office Action mailed March 23, 2010, p. 6)

Applicant respectfully disagrees.

A *prima facie* case of obviousness based on the rationale that the invention could have been achieved by "combining prior art elements according to known methods to yield predictable results" is supportable by a showing that "all the claimed elements were known in the prior art and one skilled in the art could have combined the elements as claimed by known methods with no change in their respective functions, and the combination yielded nothing more than predictable results to one of ordinary skill in the art." *See* MPEP §2143, citing the U.S. Supreme Court decision in *KSR International Co. v. Teleflex Inc.*, 550 U.S. 398 (2007); (emphasis added). It is well established that "[i]f the proposed modification or combination of the prior art would change the principle of operation of the prior art invention being modified, then the teachings of the references are not sufficient to render the claims *prima facie* obvious." (*See* MPEP 2143.01, citing *In re Ratti*, 270 F.2d 810, 123 USPQ 349 (CCPA 1959).)

Applicant points out that the combination of Ruan et al. 1999 and Van Rooijen et al. would produce a combination with a changed function and no utility in the operation of the prior art invention, and therefore provides no basis for any *prima facie* obviousness.

As amended, Applicant's claims recite a fusion protein (claims 7 and 9-11), a nucleic acid construct encoding a fusion protein (claims 1, 3, 4, 6, 12, and 46-49) and method for producing the fusion protein (claims 13, 15, 16 and 17). The fusion protein itself is recited in Applicant's amended claim 7, set forth in Section II above. As amended, the fusion protein comprises: fusion protein comprising a target protein operatively linked to the C-terminus of a subtilisin prodomain protein, wherein the subtilisin prodomain protein is modified to exhibit an increased affinity for subtilisin or a variant thereof, as compared to an unmodified subtilisin prodomain protein,

wherein the subtilisin or variant thereof is effective to cleave the target protein from the subtilisin prodomain protein, and wherein the subtilisin prodomain protein remains bound to the subtilisin or variant thereof following cleavage of the target protein.

As previously detailed in the Response mailed August 12, 2008, Van Rooijen et al. provide a fusion protein where the pro-peptide is involved in only a transient association with an enzyme, in order to allow cleavage of the fusion protein and freeing of a protein of interest and where it is a necessary characteristic of the fusion protein that after cleavage the pro-peptide disassociate from the enzyme, to allow the same enzyme to associate with and cleave additional fusion proteins.

A specific example of this function is shown in Example 4 of Van Rooijen et al. and Table 2, which lists the results of Example 4. In that Example, a GST-cystatin fusion protein is generated, with chymosin pro-peptides inserted in various locations to form different fusion proteins (shown in Figs. 13-18: GST-KLIP4-Cystatin, GST-KLIP11-Cystatin, GST-KLIP12-Cystatin, GST-KLIP14-Cystatin, GST-KLIP15-Cystatin, and GST-KLIP16-Cystatin). Chymosin is added to each of the fusion proteins and cleaves the fusion protein at the pro-peptide sites. The chymosin does not bind to the KLIP pro-peptide with high affinity, but associates only transiently as is typical of enzyme-substrate-product interactions. This allows release of chymosin after cleavage, where it is free to cleave additional fusion proteins. The results of this action are what are shown in Table 2. It is clear from the results (with the exception of KLIP12) that as long as the reaction is allowed to proceed, the chymosin continues to react with additional fusion peptides in the solution and more free cystatin is generated.

This example of Van Rooijen et al. demonstrates the necessary "catalytic turnover" of the fusion protein of Van Rooijen et al. In order to continue such catalytic turnover, the pro-peptide of the fusion protein of Van Rooijen et al. must have low affinity for the enzyme, in order to allow the disassociation of the pro-peptide and the enzyme, to permit continued accumulation of the protein of interest.

In Van Rooijen et al., the pro-peptides may be "any pro-peptide derived from any autocatalytically maturing zymogen" (col. 9, ll. 31-53). Further, mutated pro-peptides are described where the purpose of the mutated pro-peptides is "to obtain specific cleavage between the pro-peptide and a heterologous protein" and to "alter the optimal conditions, such as

temperature, pH and salt concentration, under which cleavage of a heterologous peptide is achieved..." (col. 10, ll. 24-50).

If one of skill in the art were to use a mutated prodomain of Ruan et al. 1999 in the fusion protein of Van Rooijen et al., it would change the principle of operation of the fusion protein of Van Rooijen et al. Specifically, the high binding affinity of the mutated prodomain of Ruan et al. 1999 would not permit autocatalytic cleavage to proceed. The subtilisin would bind to the modified prodomain (with a Kd of 10 nM or less) and **would remain bound**. Even once the protein of interest is cleaved, the prodomain would remain bound to the subtilisin and no additional subtilisin would be generated to bind to and cleave additional fusion proteins.

It is clear from the results in Van Rooijen et al. (with the exception of KLIP12) that the longer the reaction is allowed to proceed, the more the chymosin continues to react with additional fusion peptides in the solution and the more free cystatin is generated. If permitted to run to completion, the limiting factor in the reaction is the amount of fusion protein.

By contrast, if one of skill in the art combined the mutated prodomain of Ruan et al. 1999 with the fusion protein of Van Rooijen et al., and permitted the reaction to run to completion, the limiting factor would be the amount of subtilisin.

Therefore, the substitution of the mutated prodomain of Ruan et al. 1999 in the fusion protein of Van Rooijen et al. would result in a fusion protein that would be regarded by one of skill in the art as having no utility whatsoever in relation to the specifically described purpose and principle of operation of Van Rooijen et al.

Thus, using the mutated prodomain of Ruan et al. 1999 in the fusion protein of Van Rooijen et al. for the function described by Van Rooijen et al. would fundamentally change the principle of the experiment of Van Rooijen et al. and would not achieve the same results. As a result, the combination of Ruan et al. 1999 and Van Rooijen et al. fails to provide any tenable basis of *prima facie* obviousness of the claimed invention.

Grøn et al. 1996 and Grøn et al. 1992, cited as demonstrating particular prodomain mutations, do not remedy the deficiencies of the combination of Ruan et al. 1999 and Van Rooijen et al.

In sum, Ruan et al. 1999 and Van Rooijen et al. in view of Grøn et al. 1996 and Grøn et al. 1992 fail to provide any logical basis for the fusion protein, nucleic acid construct or methods recited in claims 1, 3, 4, 6, 7, 11-13, 17 and 46-49. Ruan et al. 1999 and Van Rooijen et al. in view of Grøn et al. 1996 and Grøn et al. 1992 do not render the claimed invention obvious. Accordingly, withdrawal of the rejection of claims 1, 3, 4, 6, 7, 11-13, 17 and 46-49 under 35 U.S.C. § 103(a) as being obvious over Ruan et al. 1999 and Van Rooijen et al. in view of Grøn et al. 1996 and Grøn et al. 1992 is respectfully requested.

CONCLUSION

All of Applicant's pending claims 1, 3, 4, 6, 7, 9-13, 15-17 and 46-49 are now patentably distinguished over the art, and in form and condition for allowance. The examiner is requested to favorably consider the foregoing, and to responsively issue a Notice of Allowance.

No fees are believed to be due for the filing of this paper. However, should any fees be required or an overpayment of fees made, please debit or credit our Deposit Account No. 08-3284, as necessary.

If any issues require further resolution, the examiner is requested to contact the undersigned attorneys at (919) 419-9350 to discuss same.

Respectfully submitted,

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